

Original Article

A bioinformatics-based approach to develop genome-editing strategies against *Helicobacter pylori* infections

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Received: 30 August 2025

Accepted: 15 December 2025

Published: 01 March 2026

DOI

10.25259/IJHS_192_2025

Quick Response Code:



**Supplementary material
available on:**

[https://dx.doi.org/10.25259/
IJHS_192_2025](https://dx.doi.org/10.25259/IJHS_192_2025)

ABSTRACT

Objectives: Antibiotic resistance in *Helicobacter pylori* (*H. pylori*) has reduced the effectiveness of standard eradication regimens, highlighting the need for alternative antimicrobial strategies. Clustered regularly interspaced short palindromic repeats (CRISPR)-CRISPR associated protein 9 (Cas9) offers sequence-specific genome targeting, but rational application requires systematic computational target prioritization.

Methods: We developed a reproducible bioinformatics framework to prioritize CRISPR-Cas9 guide Ribonucleic acids (RNAs) (gRNAs) targeting conserved core fitness genes (*recA*, *ureA*) and virulence- or colonization-associated genes (*cagA*, *vacA*, *flgE*) in *H. pylori*. Guide design, thermodynamic stability assessment, off-target screening, gene conservation analysis, and pathway/network association analyses were performed.

Results: Twelve high-confidence gRNAs met predefined efficiency and specificity criteria. Core fitness genes exhibited higher sequence conservation than virulence-associated genes. Pathway and interaction network analyses provided a qualitative context for comparative target prioritization.

Conclusion: This study presents a computational framework for prioritizing CRISPR-Cas9 targets in *H. pylori*. The findings are hypothesis-generating and intended to guide future experimental validation rather than demonstrate therapeutic feasibility.

Keywords: Antibiotic resistance, Clustered regularly interspaced short palindromic repeats (CRISPR)-associated protein 9, Guide RNA design, *Helicobacter pylori*

INTRODUCTION

Helicobacter pylori (*H. pylori*) exists as a microaerophilic Gram-negative spiral-shaped bacterium that establishes residence in human gastric tissue.^[1,2] Research indicates *H. pylori* affects more than half of the global human population and shows the highest prevalence rates in developing countries.^[3] The gastrointestinal infections caused by *H. pylori* result in chronic gastritis and peptic ulcers, mucosa-associated lymphoid tissue lymphoma, and gastric adenocarcinoma.^[4] The pathogenicity of *H. pylori* depends on virulence factors, including *cagA*, *vacA*, and *ureA*, that enable the bacterium to evade host defenses while damaging gastric epithelial cells.^[5] The standard treatment for *H. pylori* infections involves antibiotic combinations that include clarithromycin with amoxicillin or metronidazole, along with proton pump inhibitors.^[6-8] The

How to cite this article: Rehman SU. A bioinformatics-based approach to develop genome-editing strategies against *Helicobacter pylori* infections. Int J Health Sci (Qassim). 2026;20:53-61. doi: 10.25259/IJHS_192_2025

effectiveness of these treatment regimens has decreased significantly because of rising antimicrobial resistance rates.^[9] The global spread of multidrug-resistant *H. pylori* strains has led to treatment failures and recurrent infections after initial treatment.^[10] A rapid need exists for new antimicrobial methods that specifically target *H. pylori* while preserving gut microbiome diversity and overcoming resistance.

The development of antimicrobial agents using clustered regularly interspaced short palindromic repeats (CRISPR) technology requires researchers to identify which bacterial genes support essential functions for survival and persistence, versus those that primarily cause disease. The vital genes *recA* and *ureA* are highly conserved across *H. pylori* strains because they provide essential functions in genome protection and acid resistance.^[9] The bacterial determinants *cagA* and *vacA* function as virulence factors that damage host tissues and modify immune responses, but they are not essential for bacterial survival because they can be absent or differ between strains. The distinction between these two types of mutations helps scientists understand how CRISPR-mediated knockouts affect bacteria, as they can either make bacteria less fit or reduce their pathogenicity, rather than causing immediate death. The bacterial adaptive immune system CRISPR-CRISPR-associated protein 9 (Cas9) has transformed molecular biology through its repurposing for genome editing.^[11] The precise nature and efficient operation, along with the programmatic flexibility of CRISPR-Cas9, make it a suitable platform for targeting specific bacterial gene sequences.^[12] The use of CRISPR-based antimicrobials to destroy pathogenic bacteria has been targeting essential and virulence-related bacterial genes.^[13] Targeting survival and pathogenicity genes in *H. pylori* shows promise as an alternative to antibiotics.^[14,15]

Research findings show that CRISPR-based antimicrobial treatments work effectively against bacterial pathogens. Citorik *et al.*^[16] demonstrated that CRISPR-Cas systems with sequence-specific targeting capabilities could eliminate *Escherichia coli*, while Bikard *et al.*^[17] used CRISPR-Cas9 to eliminate *Staphylococcus aureus*. Holds promise for the development of targeted antibacterial treatments. Our major pathogen has widespread antibiotic resistance. The research develops a computational CRISPR-Cas9 system to eliminate

H. pylori. A whole-genome sequence analysis identified specific essential genetic loci that could serve as targets for CRISPR-based interventions. The researchers systematically generated guide ribonucleic acids (RNAs) and evaluated their specificity, efficiency, and thermodynamic properties. The research aims to develop an essential bioinformatics framework to guide laboratory-based CRISPR-based antimicrobial development against *H. pylori*. A targeted therapeutic strategy would establish a new model for the emergence of more exact medicines with a lower risk of antibiotic resistance.

MATERIALS & METHODS

Genome retrieval and analysis

We obtained the complete genome sequence of *H. pylori* strain 26695 from the National Centre for Biotechnology Information (NCBI) GenBank database in FAST-All sequence format. The OrthoFinder and BLASTn tools enabled comparative genomic analysis to identify conserved and essential genes across various clinical and reference strains. The essential genes were identified by matching the database of essential genes (DEG) database entries with established scientific literature. The CRISPR-Cas9 targeting strategy focused on critical genes involved in colonization, virulence, and survival mechanisms, including *cagA*, *vacA*, *ureA*, *recA*, and *flgE* [Table 1].

Gene conservation analysis across *H. pylori* strains

The analysis of gene conservation used 78 *H. pylori* genomes obtained from the NCBI RefSeq database. The researchers selected genome samples from different geographic origins and evolutionary relationships, avoiding both fragmented genome sequences and low-quality draft assemblies. The research team extracted coding sequences for each target gene (*recA*, *ureA*, *cagA*, *vacA*, *flgE*) from RefSeq annotations, then performed MAFFT v7 alignment with its default nucleotide settings. The researchers calculated conservation through mean pairwise nucleotide identity (%), which they measured between coding regions of the study strains and the reference strain *H. pylori* 26695 (NC_000915.1). The values

Table 1: Selected essential and virulence genes targeted by gRNAs.

Gene	Function	gRNA Sequence	PAM	On-Target Score	GC %	Off-target sites
<i>cagA</i>	Cytotoxin-associated antigen	GAGTCTGATGACCTGGAATG	NGG	82.5	55	0
<i>vacA</i>	Vacuolating cytotoxin	CTTGAGCGTCTTGAACGTTG	NGG	88.1	50	0
<i>Urea</i>	Urease enzyme subunit A	AAGTCGTGCTGATAGACCTT	NGG	79.6	45	1
<i>flgE</i>	Flagellar hook protein	CGTAGTCCAGTACCTTGGAC	NGG	84.3	60	0
<i>recA</i>	DNA repair protein	TCCGATGTTAGGACAGCTTA	NGG	90.0	50	0

gRNA: Guide RNA, PAM: Protospacer Adjacent Motif, GC: Percentage of guanine and cytosine nucleotides in the guide RNA sequence

used for conservation purposes depend on the degree of sequence similarity between genes rather than their essential functional roles. The level of gene conservation determines how well guides transfer between strains: genes with high conservation tend to have better guide portability, whereas genes with variable lineages require custom optimization for each strain.

Off-target analysis and parameter settings

The Cas-OFFinder tool helped researchers identify potential off-target sites affecting all their selected guide RNAs (gRNA) sequences. The research used two databases for its analysis: The *H. pylori* strain 26695 genome (NCBI RefSeq NC_000915.1) and the human reference genome GRCh38/hg38. The researchers conducted all their analyses using the canonical SpCas9 PAM sequence, NGG. The search for off-target sites enabled the gRNA to match potential targets with 0–4 mismatches. The program generated separate lists of off-target candidates for each gRNA, which included both exact matches (0 mismatches) and candidates with 1–4 mismatches. The analysis did not identify any predicted off-target sites that matched the *H. pylori* genome at ≤ 2 mismatches or the human genome at ≤ 3 Mismatches. The complete off-target count analysis by mismatch level appears in Table S1.

gRNA design and target site selection

The reference genome and target sequences used for gRNA design were based on *H. pylori* strain 26695 (NCBI RefSeq accession NC_000915.1). The research team obtained target gene sequences from the annotated reference genome database. The design tools and PAM system: The CHOPCHOP tool (SpCas9, NGG PAM) generated candidate guides, which CRISPOR independently evaluated. Predefined selection thresholds: The authors applied these predefined criteria to their guide selection process: (i) The Guanine (G) and Cytosine (C) content of the guides needed to fall between 40% and 60%, (ii) The CHOPCHOP efficiency score needed to reach at least 70%, (iii) The CRISPOR specificity score needed to exceed 50, (iv) The *H. pylori* genome required no off-target sites with fewer than two mismatched bases, and (v)

The human genome (GRCh38) needed to show no off-target sites with three or fewer mismatched bases. Final guide set: The evaluation process identified 12 high-confidence gRNAs from 76 initial candidate guides that met all established thresholds. The complete guide sequences along with their genomic coordinates (start/end), strand orientation, PAM information, tool performance scores, GC percentage values, and filtering results are available in Table S2.

Evaluation of gRNA efficiency and specificity

The computer evaluation process for each gRNA candidate included GC content assessment, secondary structure evaluation, and off-target site prediction using the RNAfold and Cas-OFFinder tools. The selection criteria focused on gRNAs with GC content ranging from 40 to 60%, minimal predicted off-target sites, and no hairpin structures. The assessment of thermodynamic parameters, including melting temperature and free energy (ΔG), was used to evaluate gRNA stability and hybridization efficacy [Table 2].

CRISPR construct modeling

No experimental genome editing or sequencing-based validation was performed in this study. References to CRISPResso2 were used solely to conceptually illustrate typical indel and frameshift outcomes that may arise from Cas9-mediated double-strand breaks, as reported in the literature. CRISPResso2 was not used to generate de novo cutting predictions or simulated editing efficiencies, and no empirical editing data were analyzed.

Validation of target accessibility and functional impact

To confirm Cas9 accessibility at the chosen target sites, we performed RNAstructure and mFold analyses on the secondary and tertiary structures of target gene regions. Gene essentiality scoring and search tool for the retrieval of interacting genes/proteins (STRING) database protein–protein interaction network analysis, together with Kyoto Encyclopedia of Genes and Genomes (KEGG) Mapper pathway disruption modeling, were used to predict the functional impact of gene knockouts. The combination of

Table 2: Thermodynamic and structural properties of gRNAs.

gRNA Sequence	ΔG (kcal/mol)	Hairpin stability	Melting temp (°C)	RNAfold score
GAGTCTGATGACCTGGAATG	-28.5	Low	68.2	-0.20
CTTGAGCGTCTTGAACGTTG	-30.2	Low	70.5	-0.32
AAGTCGTGCTGATAGACCTT	-25.7	Moderate	64.7	-0.18
CGTAGTCCAGTACCTTGGAC	-32.1	Low	72.1	-0.35
TCCGATGTTAGGACAGCTTA	-29.4	Low	69.3	-0.25

gRNAs: Guide RNAs

analysis levels established trust in the CRISPR-Cas9 gene disruption approach as an antimicrobial treatment.

Qualitative functional impact prioritization

The assessment of functional impact used qualitative methods to determine priorities rather than quantitatively measuring biological effects. The researchers obtained gene impact rankings by combining three evidence types: Biological function information from scientific studies, KEGG Mapper pathway association data, and STRING protein-protein interaction (PPI) network connectivity measurements. The evaluation team used a five-level ordinal scale to rank target genes by qualitative impact, with very high, high, moderate, low, and minimal categories for comparison. The study did not use any numerical weighting system; instead, it conducted experimental validation. The rankings serve as approximate indicators of the relative importance of system functions rather than actual severity levels or predicted fatal outcomes.

RESULTS

Identification of essential and virulence genes

The genomic comparison identified 43 essential genes conserved across *H. pylori* strains. The CRISPR-Cas9 system was employed to target *cagA*, *vacA*, *ureA*, *flgE*, and *recA*, as these are important virulence genes that play essential roles in colonization, immune evasion, and deoxyribonucleic acid (DNA) repair. The DEG database confirmed the essentiality of core fitness genes such as *recA* and *ureA*, whereas *cagA*, *vacA*, and *flgE* were included due to their established roles in virulence and colonization rather than strict survival requirements [Table 3].

Off-target assessment

The Cas-OFFinder tool showed that all 12 final gRNAs lacked predicted off-target sites that matched the *H. pylori*

genome with ≤ 2 mismatches and the human genome with ≤ 3 Mismatches when using the NGG PAM. The analysis at four mismatch levels revealed few theoretical human genomic matches that would not be cleaved, as Cas9 binding efficiency decreases. The complete off-target analysis results appear in Table S1.

gRNA design and optimization

The researchers created 76 candidate gRNAs spanning the five target locations. The analysis used predefined thresholds to select 12 high-confidence gRNAs, which were then further analyzed. The complete final guide set includes genomic coordinates, strand orientation, PAM and tool scores, GC%, and stability metrics, as shown in Table S1 and Figure 1.

Thermodynamic and structural evaluation

RNAfold and mFold analyses showed that the selected gRNAs were stable because they had free energy values of -22 to -32 kcal/mol. The minimum hairpin formation and stable loop structures are indicators of effective hybridization potential with target DNA sequences. The target site accessibility analysis revealed minimal secondary structure in the mRNA of the selected gene regions, which is conducive to Cas9 binding and cleavage [Figure 2].

Predicted functional impact

The researchers performed a qualitative functional impact assessment by evaluating gene functions, their roles in

Gene	Category	Conservation (%)	Functional role
<i>cagA</i>	Virulence-associated	~90	Host signaling manipulation
<i>vacA</i>	Virulence-associated	~92	Cytotoxicity and immune modulation
<i>ureA</i>	Core fitness	≥ 95	Acid resistance and gastric survival
<i>flgE</i>	Colonization-associated	~90	Motility and tissue penetration
<i>recA</i>	Core fitness	≥ 95	DNA repair and genome stability

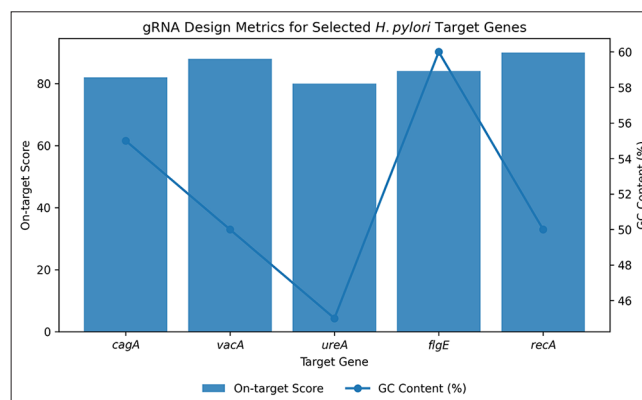


Figure 1: Distribution of candidate Clustered regularly interspaced short palindromic repeats - CRISPR associated protein 9 guide Ribonucleic acids (gRNA) target sites across selected *Helicobacter pylori* genes. The figure illustrates the relative positions of high-confidence gRNAs following computational filtering based on efficiency, specificity, and stability criteria. This visualization supports guide selection and does not represent experimental editing outcomes.

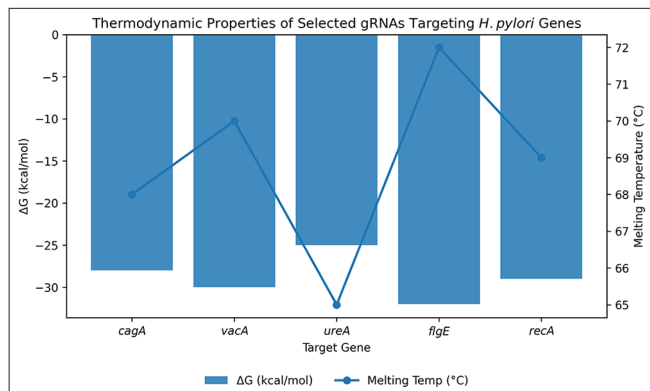


Figure 2: Predicted secondary structures of selected guide ribonucleic acids generated using ribonucleic acidfold and mFold. Structures are shown to illustrate relative stability and the absence of strong inhibitory hairpins that could affect clustered regularly interspaced short palindromic repeats associated protein 9 binding. These predictions are computational and do not reflect empirical measurements.

biological pathways, and their positions within network structures. The core fitness genes *recA* and *ureA* received very high-impact rankings because they maintain genome stability and confer acid resistance. The virulence-associated genes *cagA* and *vacA* received high impact ratings because they play essential roles in pathogen-host interactions instead of affecting bacterial survival. The colonization-associated gene *flgE* received a moderate impact rating because it helps bacteria move and create their own ecological spaces. The rankings help users select their targets, but they do not predict the extent of functional deterioration [Figure 3].

Gene conservation

The analysis of 78 *H. pylori* genomes revealed that core fitness genes exhibited patterns distinct from those of virulence-associated genes. The fitness genes *recA* and *ureA* showed greater than 95% mean nucleotide identity because they function as genome protectors and acid-resistance mechanisms. The virulence-associated gene *cagA*, along with other virulence genes, showed greater sequence diversity across lineages because these genes occur in different forms across geographic regions. The colonization-associated gene *flgE* showed a level of preservation comparable to that of the most and least conserved genes. The research indicates that CRISPR guide portability works better for core fitness genes, which have conserved sequences, but virulence genes require optimization for specific bacterial lineages or strains [Table S3 and Figure 4].

Simulated genome editing and functional disruption

The selected gRNA target sites are located at expected genomic positions, where Cas9-mediated cleavage can

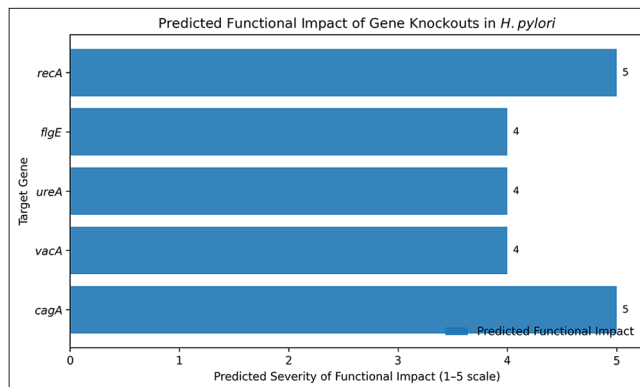


Figure 3: Search tool for the retrieval of interacting genes/proteins based protein-protein interaction networks illustrating predicted interaction partners of the selected target genes. *In silico* removal of individual nodes is shown to visualize loss of predicted interactions upon node removal, highlighting potential network vulnerability. These network representations are intended for qualitative prioritization and do not demonstrate cellular dysfunction or lethality.

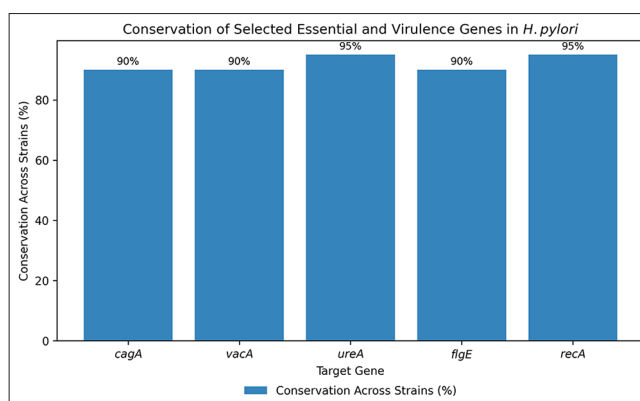


Figure 4: Conservation analysis of selected target genes across 78 *Helicobacter pylori* genomes, expressed as mean nucleotide identity relative to the reference strain. Higher conservation suggests improved guide portability but does not imply functional indispensability or resistance-proof targeting.

produce insertion-deletion mutations that could disrupt coding sequences. The established properties of non-homologous end joining after Cas9 cleavage provide the basis for this expectation, which we present as a theoretical concept rather than experimental or simulated data [Table 4].

RNAfold versus mFold

The simulated evaluation of candidate gRNA secondary structure stability through RNAfold and mFold demonstrates how these tools predict the structure of these molecules. The gRNA exhibited a stable secondary structure with minimal interfering hairpins, as determined by RNAfold analysis, suggesting efficient binding to the target DNA sequence. The

Table 4: Qualitative functional impact prioritization of CRISPR targets.

Target gene	Gene category	Primary biological role	Qualitative impact rank	Rationale
<i>recA</i>	Core fitness	DNA repair, genome stability	Very high	Essential for survival under stress; central network hub
<i>ureA</i>	Core fitness	Acid resistance	Very high	Required for gastric persistence
<i>cagA</i>	Virulence-associated	Host signaling manipulation	High	Key pathogenic effector; strain-variable
<i>vacA</i>	Virulence-associated	Cytotoxicity, immune modulation	High	Major toxin influencing disease severity
<i>flgE</i>	Colonization-associated	Motility	Moderate	Facilitates colonization but not viability

CRISPR: Clustered regularly interspaced short palindromic repeats, DNA: Deoxyribonucleic acid

mFold prediction indicates lower stability, suggesting the presence of loops or folds that could block Cas9 accessibility. Multiple structural prediction tools should be used, as stable secondary structures both improve genome-editing efficiency and reduce off-target effects [Figure 5].

KEGG pathway disruption

The researchers used KEGG pathway mapping to predict how CRISPR-mediated disruption of each target gene would affect downstream cellular processes. The research indicates that *cagA* and *recA* function as key elements that connect distinct biological networks that control host-pathogen interactions and DNA repair mechanisms; thus, their disruption would cause multiple system failures. Disruption of the *ureA* gene expression will affect acid resistance pathways that bacteria need to survive stomach acid. The model predicts which pathways will experience changes, but these predictions need experimental verification to become confirmed results [Figure 6].

STRING network pre- and post-disruption

The STRING PPI network analysis evaluated the essentiality of target genes within the *H. pylori* cellular structure. The pre-disruption network shows that proteins that control virulence, motility, and genome maintenance are interconnected. The removal of essential hub genes, including *cagA* and *recA*, through *in silico* analysis reduces network connections, indicating the system becomes more susceptible to failure. The research shows that targeting multiple vital nodes that also function as virulence factors yields better antimicrobial results. The network changes this study predicts do not prove bacterial death, as they exist only as theoretical models. The selected genes were analyzed using STRING interaction network visualization and KEGG pathway analysis to understand their positions within established biological systems. The research findings present expected interaction patterns and predicted pathway connections that can help scientists choose their most relevant targets. The visualizations serve as hypothesis-generating tools and do not provide evidence of functional impairment, cellular failure, or therapeutic effectiveness [Figure 7].

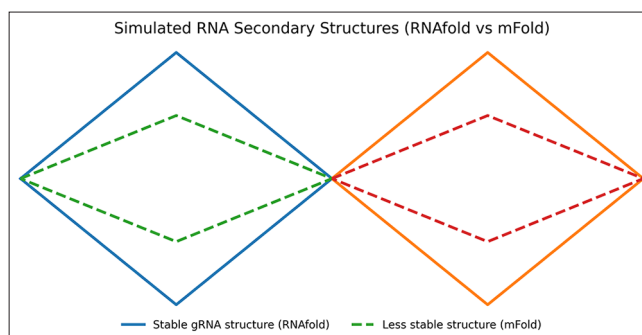


Figure 5: Kyoto encyclopedia of genes and genomes pathway mapping illustrating biological pathways associated with each target gene. The figure highlights anticipated functional linkages based on curated pathway annotations and is intended for interpretive context rather than validation of pathway impairment.

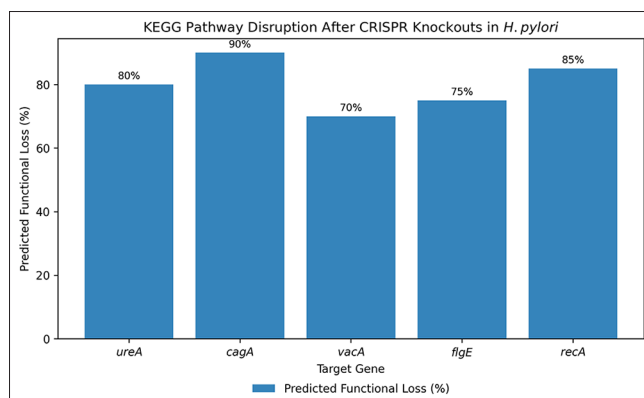


Figure 6: Summary of qualitative functional prioritization integrating gene conservation, pathway association breadth, and network connectivity. Rankings represent heuristic prioritization categories and do not correspond to quantitative measures of biological impact.

Disruption of core fitness genes *recA* and *ureA* will impair genome maintenance and acid tolerance, decreasing bacterial fitness and its ability to persist. The prediction shows that targeting virulence-associated genes (*cagA* and *vacA*) will reduce pathogen-host interactions and disease-related

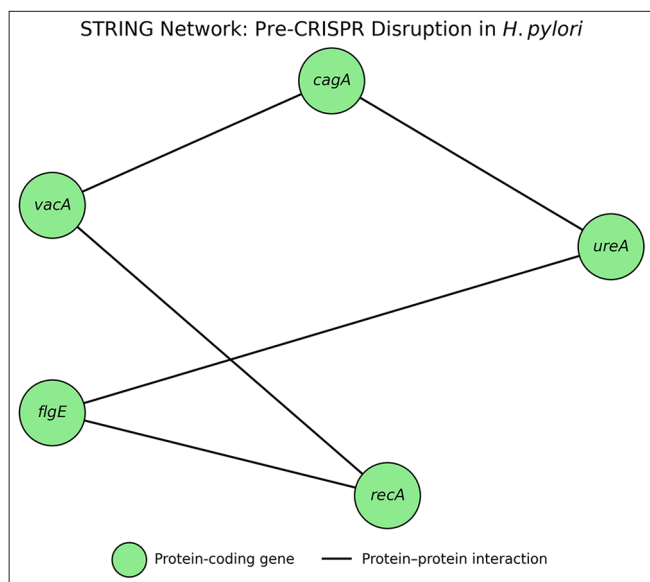


Figure 7: Search tool for the retrieval of interacting genes/proteins based protein-protein interaction networks illustrating predicted interaction partners of the selected *Helicobacter pylori* target genes. The pre-disruption network depicts known and predicted functional associations curated from experimental data, databases, and computational inference. The post-disruption visualization represents an *in silico* removal of selected target nodes to illustrate the resulting loss of predicted interactions on node removal, highlighting relative network centrality and potential system vulnerability. These networks are presented for qualitative target prioritization and contextual interpretation only and do not demonstrate functional impairment, cellular failure, or bacterial lethality.

signaling pathways without harming bacterial survival. The study includes two gene classes to develop a multi-target CRISPR approach, which will decrease pathogenicity while making it harder for bacteria to adapt.

DISCUSSION

The research establishes an extensive computer-based system that enables scientists to develop CRISPR-Cas9 gene-editing methods targeting vital *H. pylori* genes that also cause disease. The research combines genome-wide analysis with gRNA optimization, structural modeling, and pathway-level inference to discover specific targets that will disrupt essential bacterial functions. The research findings are computational results aimed at identifying potential targets for experimental studies, rather than demonstrating how the system functions or the treatment's effectiveness. Importantly, no genome editing simulations or experimental editing data were generated in this study, and all references to editing outcomes reflect established mechanistic expectations of Cas9 activity rather than direct evidence. The computational design of the CRISPR-Cas9 system for targeting *H. pylori* will make a

promising contribution to the creation of future antibacterial therapies.^[17] Our research demonstrated that gRNAs could target these genes with high efficiency and specificity.^[18] While *recA* and *ureA* represent core fitness targets aimed at reducing bacterial viability, *cagA* and *vacA* were included as virulence-focused targets intended to attenuate pathogenicity rather than eliminate bacterial survival.^[19] The study's main advantage stems from its comprehensive validation process, which includes genomic screening, gRNA optimization, structural analysis, and pathway impact evaluation. The use of *in silico* methods provides a fast, economical screening and wet-laboratory testing, and reduces the risk of future experiments.^[20] The absence of predicted off-target effects in the human genome supports the therapeutic potential of this system.

Future work needs to demonstrate CRISPR efficacy through *in vitro* and *in vivo* experiments to establish both delivery efficiency and biosafety.^[21] The two significant issues of CRISPR construct delivery across the gastric mucosa and immune system avoidance need to be addressed.^[22] Viral vectors, liposomal nanoparticles, or engineered phage systems can be used for delivering the CRISPR payload to infected *H. pylori* gastric tissues.^[23] The study establishes a robust computational framework for future experimental CRISPR-based targeting of *H. pylori*, including the identification of specific gRNA candidates and genetic loci. Precision genome-editing approaches, including this method, could revolutionize antimicrobial therapy by offering precise treatment options with minimal collateral damage to the host microbiota and by enabling the targeted treatment of antibiotic resistance.

The research study investigated multiple gene targets, but scientists need to determine which genes are vital for *H. pylori* survival. The primary function of virulence-associated genes, including *cagA* and *vacA*, is to cause severe disease symptoms that result in host tissue destruction. The core fitness genes *recA* and *ureA* enable bacteria to survive in the hostile gastric environment. The distinction between these two approaches demonstrates why researchers should use a single CRISPR targeting method that combines virulence reduction with fitness reduction rather than lethal gene-editing methods. The core fitness genes show strong conservation across species, whereas the virulence-associated gene *cagA* shows significant differences across genetic lineages. The results indicate that CRISPR guide portability for these specific loci needs individual validation for each strain, and scientists should approach statements about reduced escape potential with scepticism.

The present study focuses on computational analysis of CRISPR-Cas9 systems for sequence-specific antimicrobial targeting, but it does not explore their actual deployment. The process of clinical application faces multiple major obstacles, including delivering CRISPR constructs to

the gastric area and maintaining their stability in acidic conditions; their ability to enter *H. pylori* cells; preventing host immune system activation; and managing differences in genetic profiles between bacterial strains. The evaluation of microbiome specificity and ecological safety requires more than computer-based simulations. The translational implications presented in this work are hypothetical cases that serve to establish a framework for upcoming experimental studies.

Limitations of the study

The computational nature of this study lays essential groundwork for *H. pylori* CRISPR-based treatments, but experimental verification is necessary. More research is needed to verify the predicted *in vitro* and *in vivo* performance of the gRNA, its specific targeting capabilities, and its functional effects. The main challenge in delivering CRISPR-Cas9 constructs to the gastric mucosa remains unresolved. The stomach acid, mucus barrier, and immune system reactions need to be addressed before scientists can apply this treatment to humans. Scientists need to develop phage-based carriers, liposomal nanoparticles, and engineered viral vectors as delivery systems to achieve safe and effective delivery of the CRISPR-Cas9 construct. In addition, pathway mapping and interaction network analyses are hypothesis-generating tools and cannot establish causality, lethality, or clinical effectiveness without supporting *in vitro* or *in vivo* validation.

Ethical and practical considerations

This computational study shows that gRNAs with designed sequences exhibit high specificity for *H. pylori*. Still, the use of CRISPR-based antimicrobials for clinical treatment requires careful evaluation of biosafety risks to the human microbiota. The CRISPR could cause unintended changes to beneficial gut bacteria, leading to microbial imbalance and dysbiosis. The delivery of CRISPR constructs to the stomach faces multiple obstacles, including the potential for transfer of resistance elements between bacteria and the triggering of adverse immune reactions against delivery vehicles, such as engineered phages or nanoparticles. The therapeutic deployment of this technology requires experimental validation of its ecological effects and precise delivery systems that maintain containment before any clinical application can proceed.

CONCLUSION

This study provides a transparent and reproducible computational framework for prioritizing CRISPR-Cas9 targets in *H. pylori*. By integrating gene conservation analysis, guide RNA design metrics, off-target screening,

and functional association mapping, the work supports rational selection of candidate targets for future experimental investigation. No claims regarding therapeutic efficacy, delivery feasibility, or clinical applicability are made. Experimental validation will be required to assess biological impact, safety, and translational relevance.

Authors' contributions: This study was conducted by a single author, who was responsible for the conceptualization, methodology, data collection, analysis, writing, and final review of the manuscript.

Ethical approval: This study involved exclusively in-silico bioinformatics analyses using publicly available genomic sequence data retrieved from the NCBI GenBank and RefSeq databases. No human participants, patient-derived samples, clinical records, animal subjects, or identifiable personal data were used or accessed at any stage of the research. Therefore, in accordance with institutional and international ethical guidelines, formal ethical approval was waived by the Institutional Ethics Committee of Ala-Too International University, Bishkek, Kyrgyzstan, as the study does not constitute human or animal subject research.

Declaration of patient consent: Patient's consent is not required as there are no patients in this study.

Financial support and sponsorship: Nil

Conflict of interest: There are no conflicts of interest.

Availability of data and material: All supporting data are provided within the manuscript and as supplementary files.

Use of artificial intelligence (AI)-assisted technology for manuscript preparation: The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript, and no images were manipulated using AI.

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